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Research Article

Phylogenetic studies on the genus *Candolleomyces* (Psathyrellaceae, Basidiomycota) occurring in the bed of the Indus River, Punjab, Pakistan, reveal three new species

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Abstract

During macrofungal surveys in 2019–2024, several specimens belonging to the family Psathyrellaceae were collected from the bed of the Indus River, Punjab, Pakistan. Phylogenetic analyses, based on ITS, LSU, and tef-1α sequences and morpho-anatomical study, confirmed the novelty and placement of three taxa in the genus *Candolleomyces*. They are described as *Candolleomyces crenatus*, *C. undulatus*, and *C. virgatus*. For distinguishing characters, *C. crenatus* has crenate cap margins, small basidiospores, and a marginate base of stipe. *Candolleomyces undulatus* has parabolic to campanulate, wavy margins, light purplish gray with a light brownish gray center of pileus, and an appendiculate, pendant annulus. *Candolleomyces virgatus* has a parabolic to plane, distinct umbo, a virgate surface of pileus, 1–7 tiers, forking lamellae, and longitudinal striation on the surface of the stipe. Currently, *Candolleomyces* comprises 60 formally recognized species worldwide. However, with the inclusion of these three species, the total number rises to 63. Detailed descriptions, a phylogenetic estimate, morphological comparisons, and illustrations are provided.

Key words: Caespitose, DNA barcoding, macrofungi, mushrooms, *Psathyrella*, systematics, terrestrial

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Introduction

Wächter and Melzer (2020) established the genus *Candolleomyces* D. Wächt. & A. Melzer (family Psathyrellaceae Locq.) to accommodate 25 species of *Psathyrella* (Fr.) Quél. It was typified through *Candolleomyces candolleanus* (Fr.) D. Wächt. & A. Melzer. The genus is characterized by small to large basidiomata, a veil that is mostly present as fibrillose, fugacious, scaly, or granulose; absence of pleurocystidia; and presence of clamp connections (Wächter and Melzer 2020). The members of the genus are mostly reported from terrestrial, lignicolous, and rarely fimicolous habitats. Genus *Candolleomyces* is separated from *Psathyrella* by phylogenetic analyses and by lacking pleurocystidia (Ahmad et al. 1997; Wächter and Melzer 2020; Asif et al. 2022;

Haqnawaz et al. 2023b; Izhar et al. 2023; Ahmad et al. 2024; Haqnawaz et al. 2024a, b). Currently, the genus comprises 60 species and shows worldwide distribution (Ahmad et al. 1997; Wächter and Melzer 2020; Asif et al. 2022; Haqnawaz et al. 2023b; Izhar et al. 2023; Nayana and Pradeep 2023a, b; Ahmad et al 2024; Han et al. 2024; Haqnawaz et al. 2024a, b). Nevertheless, Asia is the richest continent for diversity of *Candolleomyces*, sporting forty-one species (68.33%), while eleven are known from Europe (18.33%), six from North America (10%), and one species each from Africa (1.66%) and South America (1.66%) (Haqnawaz et al. 2024a, b). From Pakistan, 14 species have been reported so far (Haqnawaz et al. 2024a, b), which corresponds to about a 34.14% contribution of the species known from Asia and 23.33% worldwide. In this study, based on the morpho-anatomical characters and phylogenetic analyses, we introduce three more new species of the genus *Candolleomyces*.

Materials and methods

Sampling site (Fig. 1)

Basidiomata of macrofungi were collected from different localities of the Indus Riverbed (30°25'21"N, 70°52'46"E) (129–139 m a.s.l.), Kot Addu, Punjab, Pakistan, during July to December 2019 to 2024. The highest temperature was 51 °C in summer, and the lowest temperature was -1 °C in winter, and the average annual rainfall is 127 mm (Ijaz et al. 2020; Haqnawaz et al. 2023a, b; Haqnawaz et al. 2024a, b). The predominant flora consists of *Vachellia nilotica* (L.) P.J.H.Hurter & Mabb., *Saccharum bengalense* Retz., *S. spontaneum* L., *Dalbergia sissoo* Roxb., *Phoenix dactylifera* L., *Tamarix aphylla* (L.) Warb., *Albizia chinensis* (Osbeck) Merrill, and *Calotropis procera* (Ait.) Ait., Hort. (Stewart 1972; Haqnawaz et al. 2024a, b).

Morphological characterization

Fresh basidiomata were collected and photographed with tags. Morphological characteristics of samples were noted following terminologies by Vellinga (2001). Color codes were noted according to the Munsell Color Chart (1994). The collected basidiomata were dried using a hot air drier at 30 °C, packed in polythene bags, and deposited at the LAH Herbarium, Institute of Botany, University of the Punjab, Quaid-e-Azam Campus, Lahore, Pakistan. Tissues from different parts of the basidiomata were rehydrated in 5% KOH and stained in Congo Red (1% w/v). Microscopic structures were examined using a compound microscope (OLYMPUS BX43, Tokyo, Japan) and measured using ScopeImage 9.0 software connected to the microscope through a digital camera (HDCE–90D). The short form [n/m/p] indicates 'n' basidiospores measured from 'm' basidiomata of 'p' collections. Basidiospore measurements were recorded as (a–) b–c (–d), where a = extreme minimum value, range b–c covers at least 90% of the calculated values, and d = extreme maximum value; 'Q' shows the individual spore length/width ratio while 'Qav' presents the average of all Q values (Ge et al. 2010).

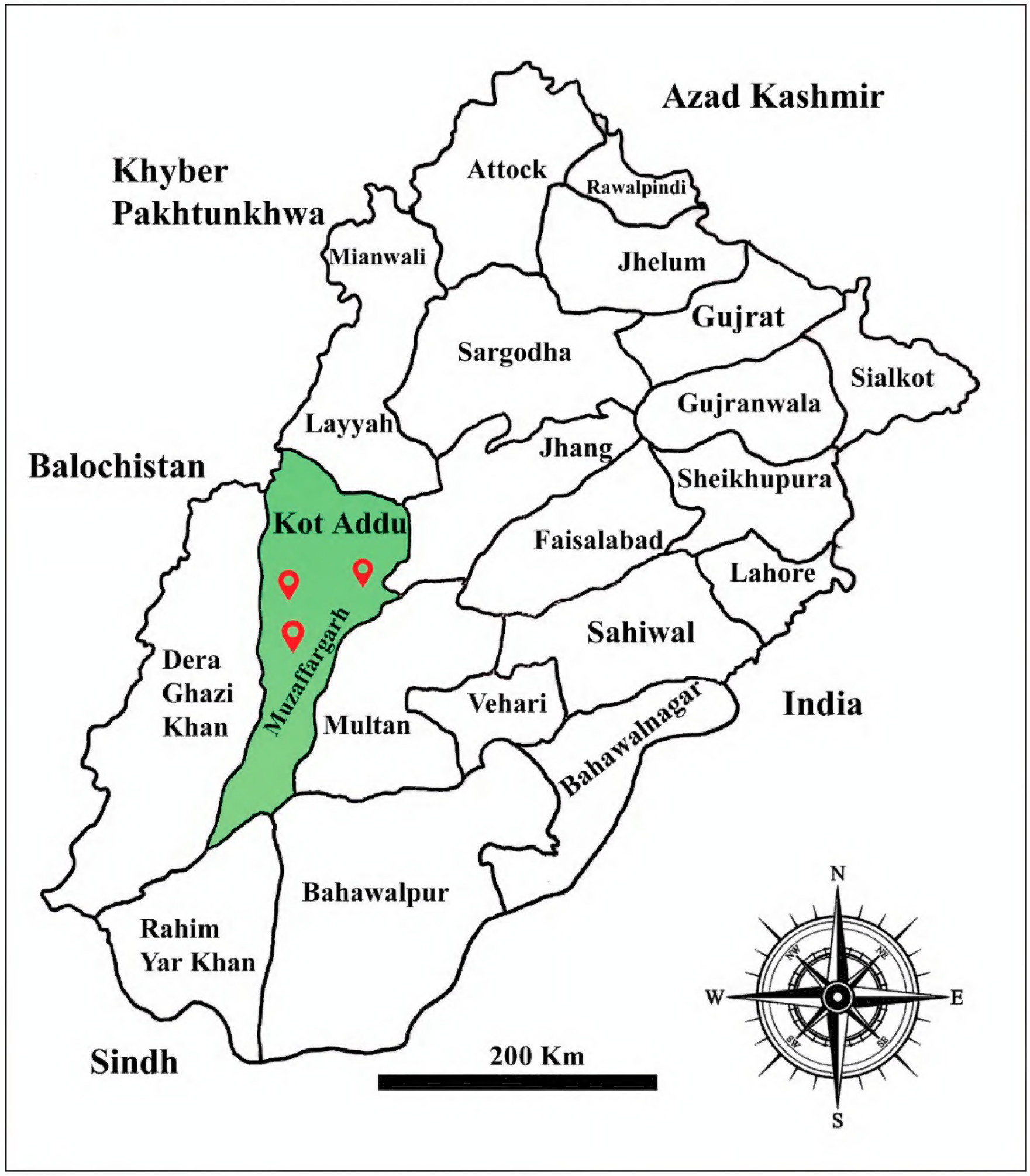

Figure 1. Map of sampling sites where the novel taxa were found, with the green color showing the district of the sampling sites.

Molecular phylogenetic analyses

Total DNA was extracted from lamellae of dried basidiomata using a modified 2% CTAB method (Bruns 1995), and the DNA quality was assessed by 1% (w/v) agarose gel electrophoresis (Voytas 2000). The ITS region of the rDNA was amplified using the ITS1F and ITS4 primers; for the LSU region, the LR0R and LR5 primers; and for the tef-1α gene, the EF1-983F and EF1-1567R primers were used (Vilgalys and Hester 1990; White et al. 1990; Gardes and Bruns 1993). The PCR procedure given by Usman and Khalid (2020)

was followed, and PCR products were then sent to the sequencing company TsingKe, China.

The final ITS, LSU, and tef-1α rRNA consensus sequences were obtained by assembling both forward and reverse primers using BioEdit V 7.2.5 (Hall 2009). Sequences used in the combined dataset (ITS-LSU- and tef-1α rRNA) were retrieved from the NCBI database GenBank (Sayers et al. 2024), based on similarity of 95% identity or greater, plus all published sequences from the genus *Candolleomyces* (Ediriweera et al. 2023; Izhar et al. 2023; Nayana and Pradeep 2023a, b; Haqnawaz et al. 2024a, b). For the phylogenetic analyses, a Clustal W MUSCLE alignment (Edgar 2004) was implemented in BioEdit V 7.2.5 with manual adjustment. A combined (ITS-LSU-tef-1α) maximum likelihood phylogenetic tree was constructed using RAxMLHPC2 v. 8.2.12 on XSEDE tool (8.2.10) on the CIPRES portal online. The GTR+GAMMA nucleotide substitution model was used, and 1000 bootstrap iterations were performed with rapid bootstrapping (Miller 2010). Bayesian inference phylogenetic analyses were performed using MrBayes v. 3.2.1 (Ronquist et al. 2012, online version). The model of evolution was estimated by MrModeltest 2.2 (Nylander 2004). The Markov Chain Monte Carlo (MCMC) sampling in MrBayes v.3.2.2 (Ronquist et al. 2012) was used to determine the posterior probabilities (PP). Six simultaneous Markov chains were run for 1,000,000 generations, and trees were sampled every 1000th generation. Bootstrap values ≥ 50% and Bayesian posterior probabilities (PP) ≥ 0.80 are mentioned on the branches that were visualized in FigTree v. 1.4.2 (Rambaut 2014). The newly generated sequences were deposited in GenBank, and short descriptions of the species were deposited in MycoBank (Robert et al. 2013).

Results

Phylogenetic analyses (Fig. 2, Table 1)

The combined phylogenetic tree was constructed based on ITS, LSU, and tef-1α regions using maximum likelihood and Bayesian inference methods. It contains 57 sequences of genus *Candolleomyces*, while the sequence of *Psathyrella thujina* A.H. Sm. was used as an outgroup. The newly proposed taxa are represented in bold in the final phylogenetic tree (Fig. 2). All three new species, viz., *Candolleomyces crenatus*, *C. undulatus*, and *C. virgatus*, formed separate branches from their closest species (Fig. 2). Our first taxon, *Candolleomyces crenatus*, makes a separate branch from *C. badiophyllus* with a difference of 17 base pairs in ITS, LSU, and tef-1α sequences. The second new species, *C. undulatus*, appears as a sister species to *C. efflorescens* (Sacc.) D. Wächt. & A. Melzer, *C. albipes* (Murrill) D. Wächt. & A. Melzer, and *C. tuberculatus* (Pat.) D. Wächt. & A. Melzer, with differences of 30, 33, and 35 base pairs in the ITS sequence, respectively. The third new species, *Candolleomyces virgatus*, is a sister species to *C. campanulatus* A. Izhar, Z. Khan & Khalid and *C. amygdaliformis* Haqnawaz, Niazi, I. Ahmad & Khalid, with differences of 31 and 13 base pairs in the ITS-LSU sequence, respectively.

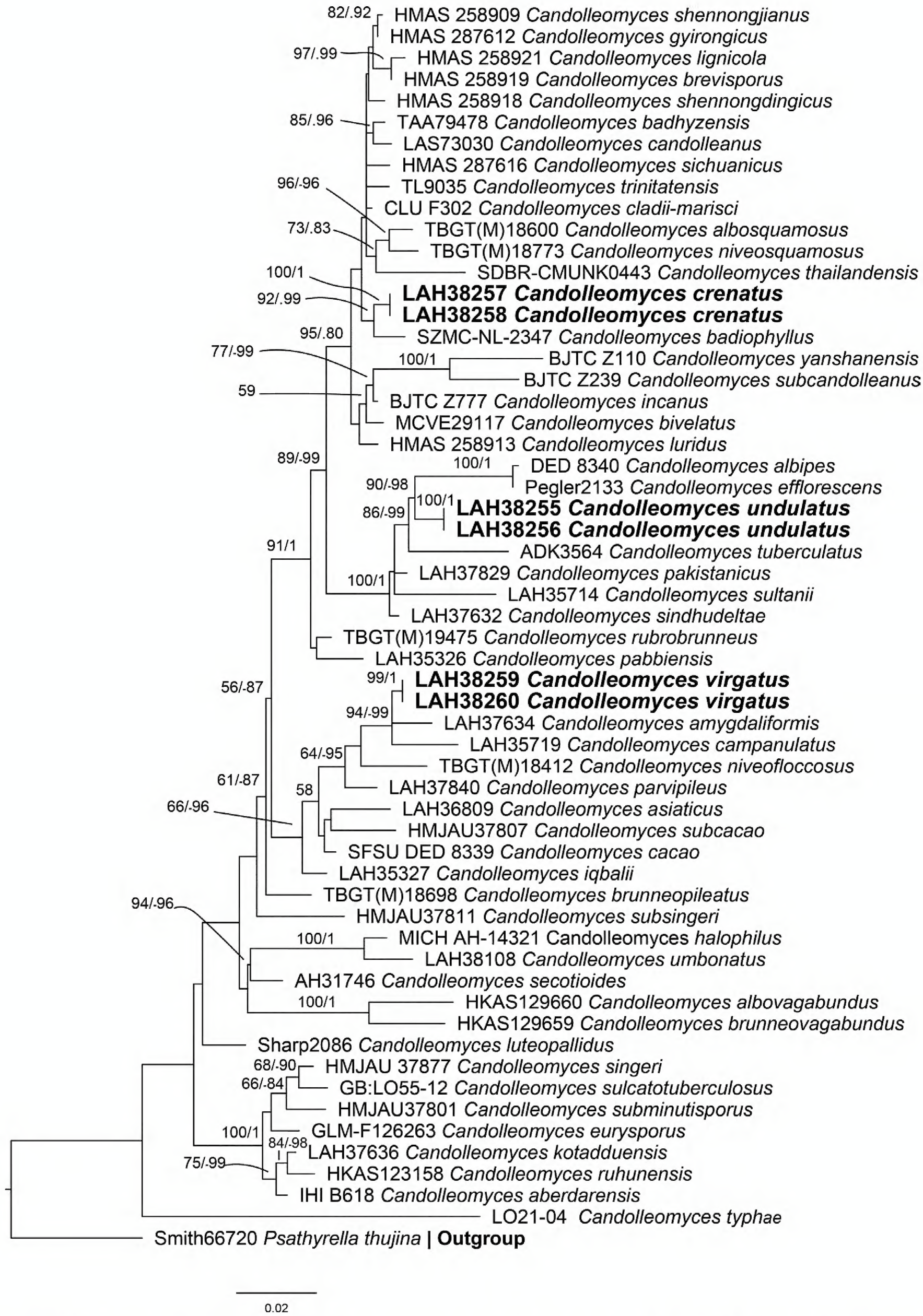

Figure 2. Phylogenetic tree of the genus *Candolleomyces* as generated by maximum likelihood (ML) and Bayesian analyses, based on combined ITS, LSU, and tef-1α sequences. Bootstrap values > 50%, based on 1,000 replicates and Bayesian posterior probabilities (PP) ≥ 0.80 are shown at the branches. Novel sequences, generated during this study, are shown in bold.

Table 1. Sequences used for phylogenetic analyses of *Candolleomyces*, including their current names, locality, voucher specimen, and GenBank accession numbers of the ITS, LSU, and tef-1α regions. Sequences generated during this study are shown in bold.

Species Name	Locality	Voucher	ITS	LSU	tef-1α
Candolleomyces aberdarensis	Kenya	IHI B618	MK421517	MK421517	-
C. amygdaliformis T	Pakistan	LAH37634	PP375293	PP375296	-
C. albipes	São Tomé	DED 8340	KX017209	-	-
C. albosquamosus T	India	TBGT(M)18600	OQ676549	-	-
C. albovagabundus T	China	HKAS129660	OR711041	OR711057	OR727285
C. asiaticus T	Pakistan	LAH36809	OK392605	OQ802842	-
C. badhyzensis T	Turkmenistan	TAA79478	KC992883	-	-
C. badiophyllus	Hungry	SZMC-NL-2347	FN430699	FM876268	FM897252
C. bivelatus	Italy	MCVE29117	MF325962	-	MF521811
C. brunneopileatus T	India	TBGT(M)18698	OQ878348	OR244401	-
C. brunneovagabundus T	China	HKAS129659	OR711031	OR711047	OR791600
C. brevisporus T	China	HMAS 258919	OR822167	OR822149	OR819986
C. cacao T	Sao Tomé	SFSU DED 8339	KX017210	-	-
C. campanulatus T	Pakistan	LAH35719	OQ308881	OQ802837	-
C. candolleanus	Sweden	LAS73030	KM030175	KM030175	-
C. cladii-marisci T	Italy	CLU F302	MK080112		-
***C. crenatus* T**	**Pakistan**	**LAH38257**	**PQ329548**	**PQ329553**	**PQ369432**
C. crenatus	**Pakistan**	**LAH38258**	**PQ329547**	**PQ329554**	**PQ369433**
C. gyirongicus T	China	HMAS 287612	PP734613	PP734624	PP729326
C. kotadduensis T	Pakistan	LAH37636	OQ968359	OQ968362	-
C. lignicola T	China	HMAS 258921	OR822169	OR822151	OR819988
C. pakistanicus T	Pakistan	LAH37829	OQ968356	OQ968363	-
C. efflorescens	Sri Lanka	Pegler2133	KC992941	-	-
C. eurysporus T	Vietnam	GLM-F126263	MT651560	MT651560	-
C. halophilus T	Spain	MICH AH-14321	MG825900	-	-
C. incanus T	China	BJTC Z777	ON042759	ON042766	ON098509
C. iqbalii T	Pakistan	LAH35327	OQ968353	OQ968366	-
C. luteopallidus T	USA	Sharp20863	KC992884	KC992884	-
C. luridus T	China	HMAS 258913	OR822160	OR822143	OR819980
C. niveofloccosus T	India	TBGT(M)18412	OQ878345	OR244387	-
C. niveosquamosus T	India	TBGT(M)18773	PP741631	PP741635	-
C. pabbiensis T	Pakistan	LAH35326	PP058339	PP058345	-
C. parvipileus T	Pakistan	LAH37840	OQ968357	OR506278	-
C. rubrobrunneus T	India	TBGT(M)19475	PP741633	PP741636	-
C. ruhunensis T	Sri Lanka	HKAS123158	ON685315	-	-
C. secotioides T	Mexico	AH31746	KR003281	KR003282	KR003283
C. shennongjianus T	China	HMAS 258909	OR822157	OR822139	OR819976
C. shennongdingicus T	China	HMAS 258918	OR822166	OR822148	OR819985
C. sindhudeltae T	Pakistan	LAH37632	OQ247908	OQ247912	-
C. singeri	China	HMJAU 37877	MW301073	MW301091	MW314080
C. subcacao T	China	HMJAU37807	MW301064	MW301092	MW314081
C. sichuanicus T	China	HMAS 287616	PP734617	PP734628	PP729330
C. subcandolleanus T	China	BJTC Z239	ON042755	ON042762	ON098505
C. subminutisporus T	China	HMJAU37801	MW301066	MW301094	MW314083
C. subsingeri T	China	HMJAU37811	MG734715	MW301097	MW314085
C. sulcatotuberculosus	Germany	GB:LO55-12	KJ138422	-	-
C. sultanii T	Pakistan	LAH35714	OQ308835	OQ801565	-
C. thailandensis T	Thailand	SDBR-CMUNK0443	MZ146874	-	-
C. trinitatensis	Ecuador	TL9035	KC992882	-	-

Species Name	Locality	Voucher	ITS	LSU	tef-1α
C. tuberculatus	Benin	ADK3564	KC992934	-	-
C. typhae	Sweden	LO21-04	DQ389721	DQ389721	KJ732776
C. umbonatus T	Pakistan	LAH38108	PP058341	PP058347	-
***C. undulatus* T**	**Pakistan**	**LAH38255**	**PQ329546**	**PQ329551**	**PQ369428**
C. undulatus	**Pakistan**	**LAH38256**	**PQ329545**	**PQ329552**	**PQ369429**
***C. virgatus* T**	**Pakistan**	**LAH38259**	**PQ329543**	**PQ329549**	**PQ369430**
C. virgatus	**Pakistan**	**LAH38260**	**PQ329544**	**PQ329550**	**PQ369431**
C. yanshanensis T	China	BJTC Z110	ON042758	ON042765	ON098507
Psathyrella thujina **(Outgroup)**	USA	Smith66720	KC992876	KC992876	-

Taxonomy

Candolleomyces crenatus Haqnawaz, Niazi & Khalid, sp. nov.

MycoBank No: 855774

Figs 3, 4

Etymology. Species name "*crenatus*" (Latin) refers to the crenate cap margins.

Holotype. Pakistan • Punjab Province: Kot Addu District, Indus Riverbed, (30°28'03"N, 70°49'25"E, 126 m a.s.l.), on loamy soil, rich in organic matter, under *Vachellia nilotica*, 07 Aug. 2022, Muhammad Haqnawaz, Q–13 (***Holotype*** LAH38257). GenBank: PQ329548 [ITS], PQ329553 [LSU], PQ369432 [tef-1α].

Diagnosis. This species is different from *Candolleomyces badiophyllus* by its convex to plane, crenate cap margins, wrinkled, dull surface, light gray, with dull orange center of pileus, marginate base of stipe, and small basidiospores (7–9 × 4–5 µm).

Description. ***Pileus*** 14–34 mm diameter, convex to plane, straight shape, and crenate cap margins; presence of veil elements; wrinkled, dull surface; light gray (7.5 YR 8/1) with dull orange (7.5YR 7/3) center. ***Lamellae*** adnexed, ventricose, average, close, fimbriate, unequal, 1–5 tiers, dull reddish brown (5 YR 5/3). ***Stipe*** 23–35 × 3–5 mm, compressed, flexuous, tomentose, marginate base. ***Annulus*** absent. Taste and odor are not recorded.

Basidiospores [150/3/2], (7–)7.5–8.5(–9) × (4–)4.5–5(–5.7) µm, Q = 1.4–1.8, Qav = 1.61, ellipsoid, thick-walled, smooth, rarely guttulate, central germpore present, reddish brown in water. ***Basidia*** (15–)16–23(–24) × (7–)7.5–11(–12) µm, clavate to broadly clavate, hyaline to yellowish in water, thick-walled, smooth, with 3–4 sterigmata. ***Cheilocystidia*** (15–)16–31(–32) × (7–)8–12(–13) µm, ellipsoid to broadly ellipsoid, clavate to broadly clavate, globose, utriform to utriform with median constriction, cylindrical, hyaline to yellowish in water, thick-walled. ***Pleurocystidia*** absent. ***Pileipellis*** an irregular epithelium, thick-walled, 18–30 × 16–24 µm, globose to subglobose and ellipsoid to broadly ellipsoid, clavate to broadly clavate cells, hyaline to light yellowish in water. ***Stipitipellis*** a cutis, hyphae subregular, branched 6–17 µm in diameter, thin-walled, septate, and hyaline to yellow in water. ***Caulocystidia*** (17–)18–42(–43) × (8–)9–14(–15) µm, utriform, clavate to broadly clavate, globose, broadly ellipsoid, hyaline in water. Clamp connections present in all tissues.

Ecology and habitat. Terrestrial, caespitose, on loamy soil rich in organic matter, under *Vachellia nilotica*.

Additional material examined. Pakistan • Punjab Province: Kot Addu, Pirhar Gherbi, (30°25'07"N, 70°52'35"E, 136 m a.s.l.), on loamy soil, 11 Aug. 2023,

Figure 3. A–D basidiomata of *Candolleomyces crenatus* sp. nov. (Holotype LAH38257). Scale bars: 10 mm.

Muhammad Haqnawaz, HA–03 (***Paratype*** LAH38258). GenBank: PQ329547 [ITS], PQ329554 [LSU], PQ369433 [tef-1α].

Candolleomyces undulatus **Haqnawaz, Niazi & Khalid, sp. nov.**
MycoBank No: 855775
Figs 5, 6

Etymology. Species name "*undulatus*" (Latin) refers to the wavy cap margins.

Holotype. Pakistan • Punjab Province: Kot Addu District, Indus Riverbed, (30°25'33"N, 70°52'12"E, 130 m a.s.l.), on loamy soil, rich in organic matter, under *Phoenix dactylifera*, 28 Aug. 2020, Muhammad Haqnawaz, KA–39 (***Holotype*** LAH38255). GenBank: PQ329546 [ITS], PQ329551 [LSU], PQ369428 [tef-1α].

Diagnosis. This species is different from its closest species by parabolic to campanulate, wavy margins, light purplish gray with light brownish gray center of pileus, appendiculate, pendant annulus, oblong to ellipsoid, globose, ovoid, cheilocystidia, and caulocystidia.

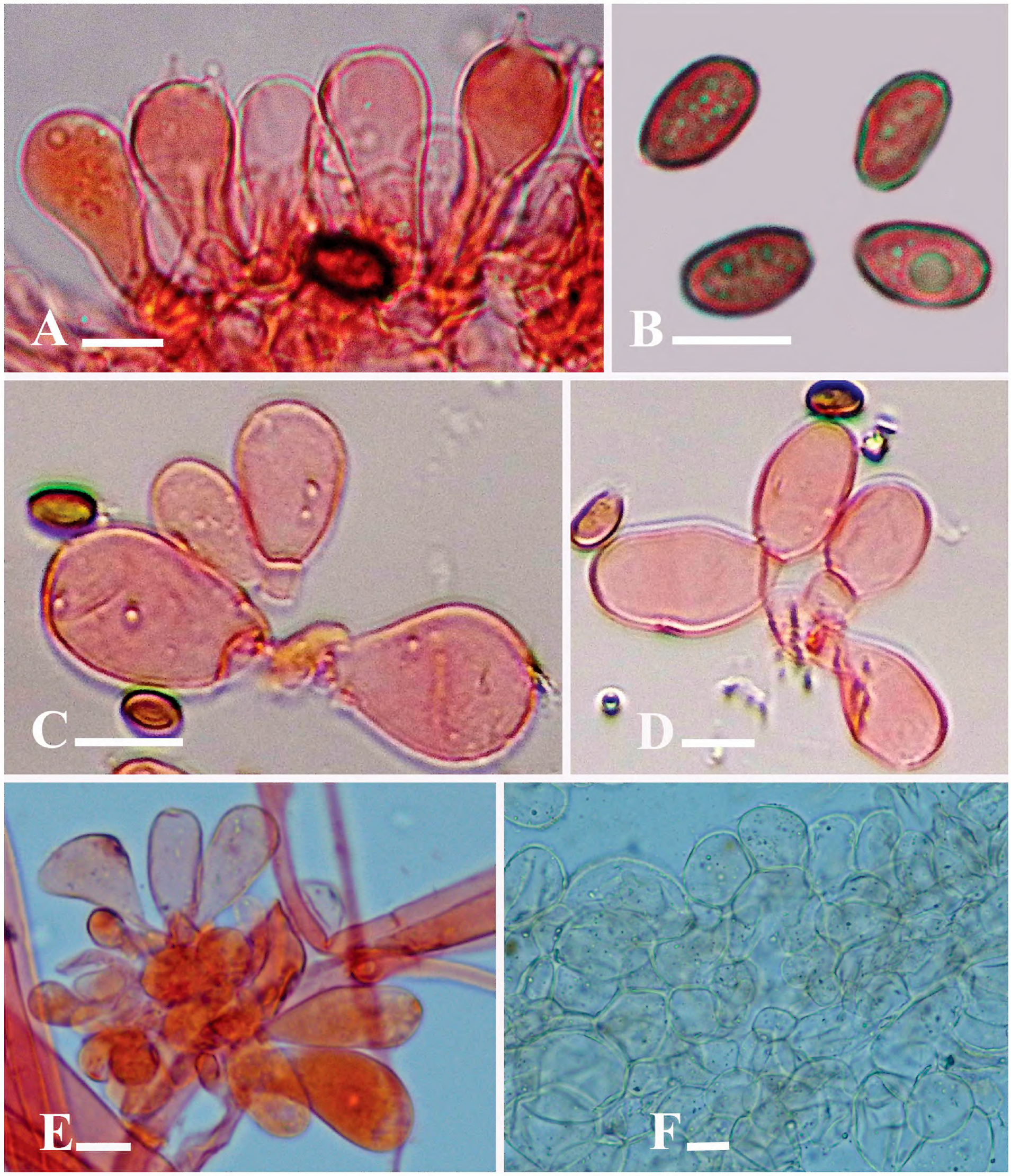

Figure 4. Microscopic structures of *Candolleomyces crenatus* sp. nov. (Holotype LAH38257) **A** basidia **B** basidiospores **C, D** cheilocystidia **E** caulocystidia **F** pileipellis. Scale bars: 10 μm (**A–F**).

Description. ***Pileus*** 10–27 mm diameter, parabolic to campanulate, straight shape and wavy cap of margins, presence of veil elements, light purplish gray (5 P 7/1), and light brownish gray (5YR 7/2) center. ***Lamellae*** adnexed to adnate, narrow, average, close, eroded, unequal, 1–3 tiers, light reddish gray (7.5 R 7/1), ***Stipe*** 15–46 × 2–4 mm, concolourous to pileus, punctate, flexuous, tomentose. ***Annulus*** appendiculate and pendant. ***Taste*** and ***odor*** not recorded.

Figure 5. A, B basidiomata of *Candolleomyces undulatus* sp. nov. (Holotype LAH38255). Scale bars: 10 mm.

Basidiospores [150/3/2], (6–)6.5–7.5(–8) × (3.5–)4–5(–6.6) µm, Q = 1.15–1.94, Qav = 1.58, ellipsoid to oblong, thick-walled, smooth, guttulate, germpore present, brownish with yellowish guttule in water. ***Basidia*** (15–)16–24(–25)

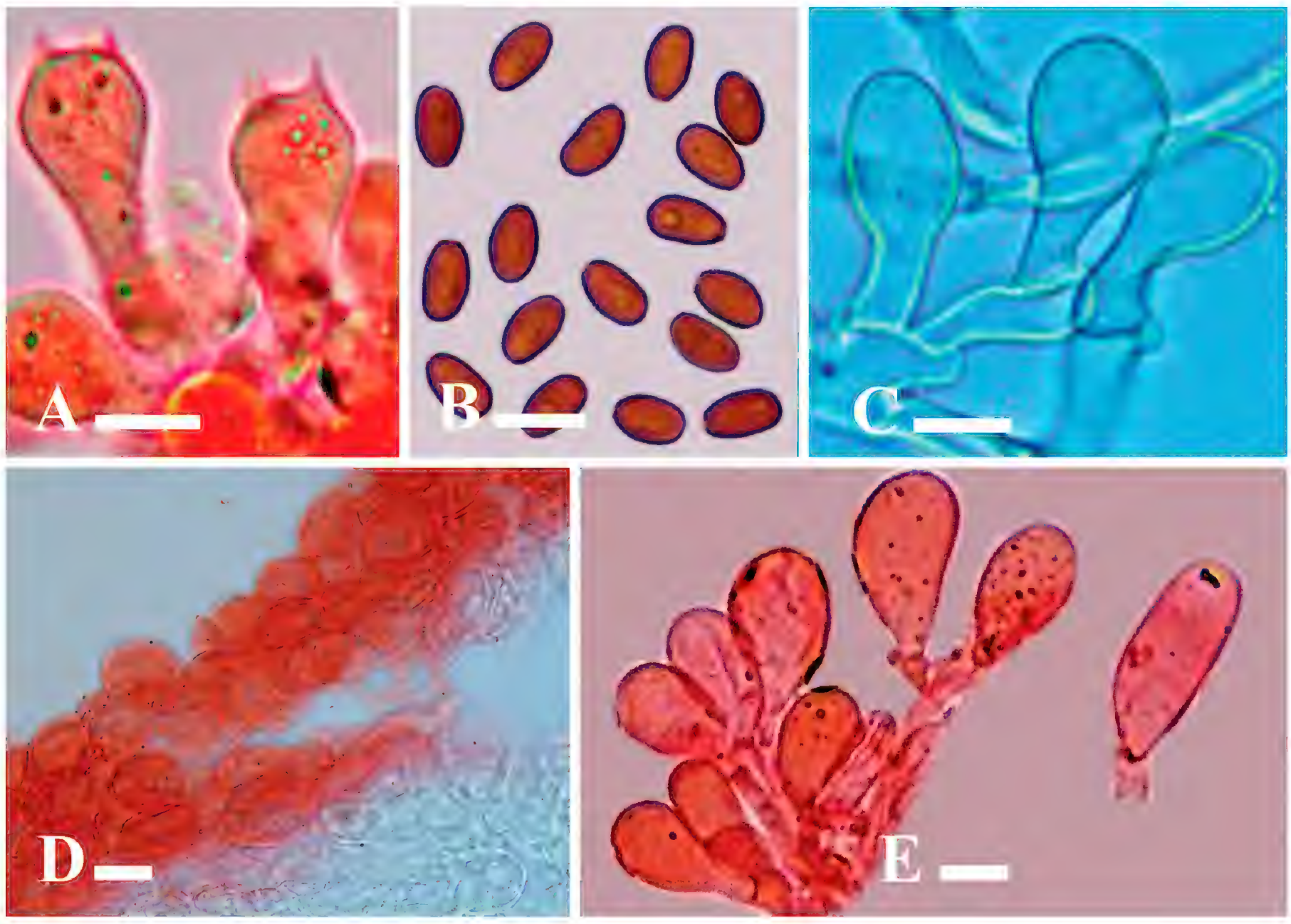

Figure 6. Microscopic structures of *Candolleomyces undulatus* sp. nov. (Holotype. LAH38255) **A** basidia **B** basidiospores **C** caulocystidia **D** pileipellis **E** cheilocystidia. Scale bars: 10 µm (**A–E**).

× (7–)7.5–11(–12) µm, clavate to broadly clavate, rarely cylindrical, spherical with broad peduncle, hyaline in water, thick-walled, smooth, with 2–4 sterigmata. ***Cheilocystidia*** (15–)16–31(–32) × (7–)8–12(–13) µm, oblong to ellipsoid, globose, ovoid, utriform, hyaline in water, thick-walled. ***Pleurocystidia*** absent. ***Pileipellis*** an irregular epithelium, thick-walled, 18–30 × 16–24 µm diameter, globose to subglobose and ellipsoid to broadly ellipsoid, hyaline in water. ***Stipitipellis*** a cutis, hyphae subregular, branched 7–18 µm in diameter, thin-walled, septate, hyaline in water. ***Caulocystidia*** 17–)18–42(–43) × (8–)9–14(–15) µm, utriform, clavate to broadly clavate, ovoid, spheropedunculate, globose, hyaline in water. Clamp connection present in all tissues.

Ecology and habitat. Terrestrial, connate, on loamy soil rich in organic matter, under *Phoenix dactylifera*.

Additional material examined. Pakistan • Punjab Province: Kot Addu, Noor Shah Thal, (30°27'39"N, 70°52'40"E, 129 m a.s.l.), on loamy soil, 11 Aug 2021, Muhammad Haqnawaz, SSC–44 (***Paratype*** LAH38256). GenBank: PQ329545 [ITS], PQ329552 [LSU], PQ369429 [tef-1α].

Candolleomyces virgatus **Haqnawaz, Niazi & Khalid, sp. nov.**
MycoBank No: 855776
Figs 7, 8

Etymology. Species name "*virgatus*" (Latin) refers to the virgate pileus surface.

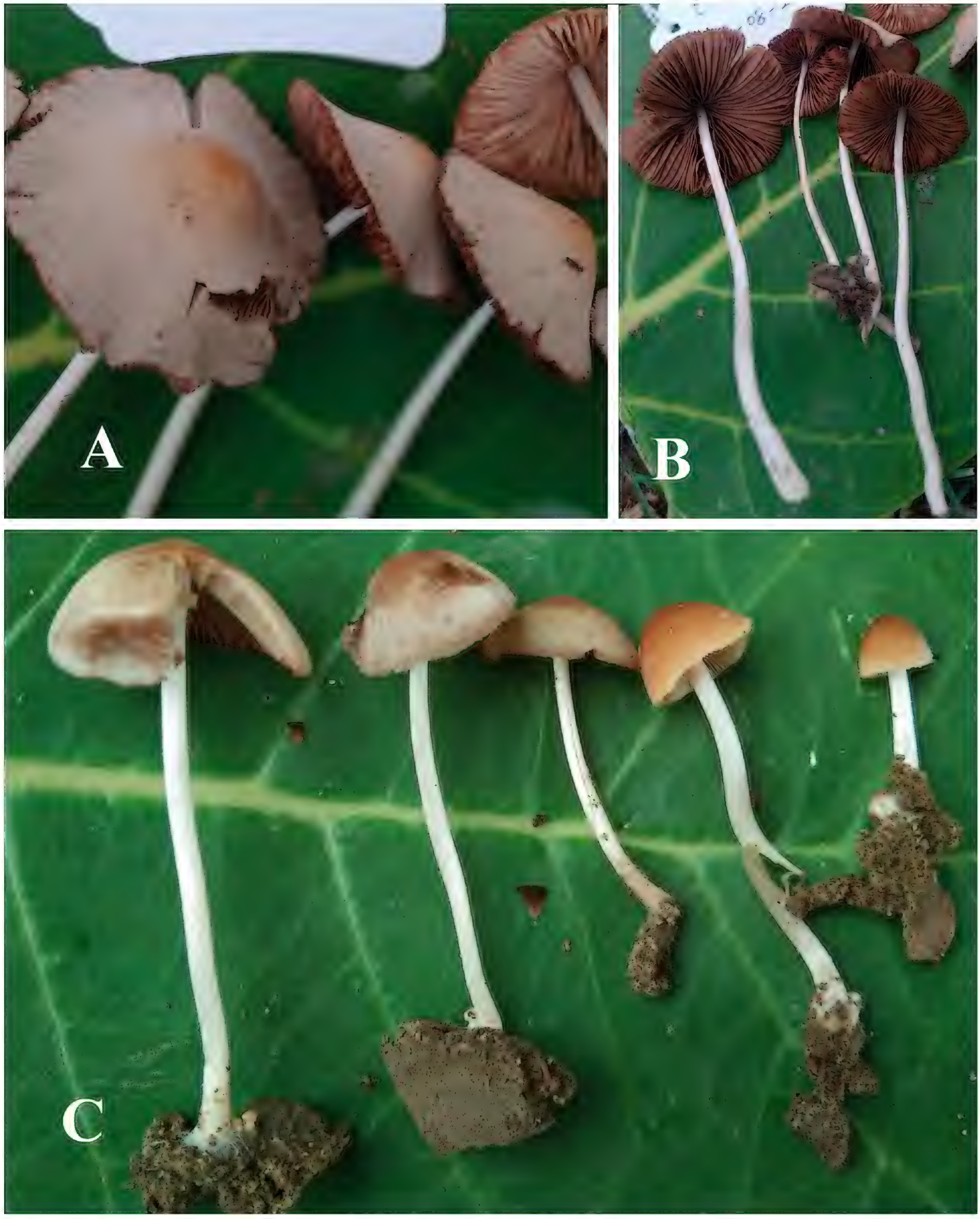

Figure 7. A–C basidiomata of *Candolleomyces virgatus* sp. nov. (Holotype LAH38259). Scale bars: 10 mm.

Holotype. Pakistan • Punjab Province: Kot Addu District, Indus River-bed, (30°14'44"N, 70°51'04"E, 134 m a.s.l.), on soil, rich in organic matter, 29 Aug 2020, Muhammad Haqnawaz, EF–17 (***Holotype*** LAH38259). GenBank: PQ329543 [ITS], PQ329549 [LSU], PQ369430 [tef-1α].

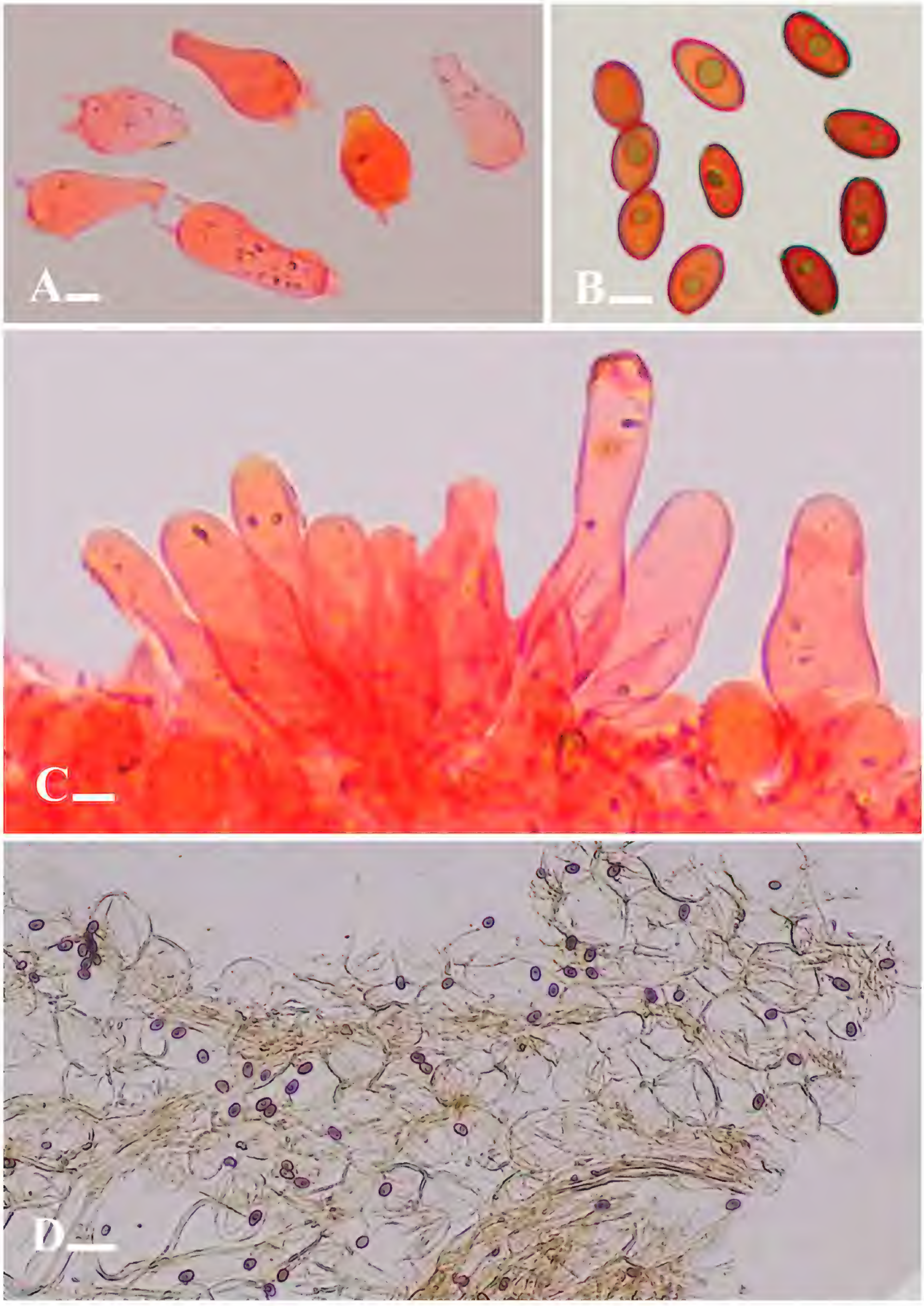

Figure 8. A–D microscopic structures of *Candolleomyces virgatus* (Holotype. LAH38259) **A** basidia **B** basidiospores **C** cheilocystidia **D** pileipellis. Scale bars: 10 µm (**A–C**); (**D**) 20 µm (**D**).

Diagnosis. This species is different from *Candolleomyces campanulatus* by its parabolic to plane, distinct umbo, virgate surface of pileus, crinkled, 1–7 tiers of forking lamellae, marginate base, longitudinally striation on surface of stipe, lageniform with moniliform at top, moniliform, cylindrical, tibiiform, conical cheilocystidia.

Description. Basidiomata medium-sized. ***Pileus*** 17–30 mm diameter, parabolic at young stage, campanulate to plane, umbonatus, straight shape and regular cap of margins, virgate, light brownish gray (7.5 YR 7/1), and dull reddish brown (5YR 5/4) center. ***Lamellae*** adnate, broad, crinkled, 1–7 tiers of lamellae, branched or forking; some are not branched, grayish red (10R 6/2). ***Stipe*** 40–80 × 1–4 mm, white gray (N 8/0), slightly flexuous, hollow, tomentose, and broad at base, marginate, longitudinally striation. Annulus absent.

Basidiospores [150/2/3], (6–)7–9(–10) × (4–)4.5–5(–6) µm, Q = 1.2–1.8, Qav = 1.61, ellipsoid to broadly ellipsoid, thick-walled, smooth, guttulate, germ pore present, purplish to brownish with greenish to yellow guttule in 5% KOH and water. ***Basidia*** (12–)13–29(–30) × (6–)7–9(–10) µm, clavate, broadly clavate, hyaline to light yellowish in 5% KOH, thick-walled, smooth, with 2–4 sterigmata. ***Cheilocystidia*** (26–)27–46(–47) × (8–)9–13(–14) µm, utriform, lageniform with moniliform at top, moniliform, cylindrical, tibiiform, conical, hyaline to light yellowish in 5% KOH. ***Pleurocystidia*** and ***Caulocystidia*** absent. ***Pileipellis*** an irregular epithelium; pileipellis cells 30–60 × 20–42 µm diameter, globose to subglobose and ellipsoid to broadly ellipsoid, clavate, hyaline to light yellowish in 5% KOH. ***Stipitipellis*** a cutis, hyphae subregular, branched 6–15 µm in diameter, thin-walled, septate, hyaline to light yellowish in 5% KOH. Clamp connection present in all tissues.

Ecology and habitat. Terrestrial, gregarious, scattered, on loamy soil rich in organic matter.

Additional material examined. Pakistan • Punjab Province: Kot Addu, Noor Shah Thal, (30°27'47"N, 71°02'05"E, 132 m a.s.l.), on loamy soil, 02 September 2020, Muhammad Haqnawaz, KA–59 (***Paratype***; LAH38260) GenBank PQ329544 [ITS], PQ329550 [LSU], PQ369431 [tef-1α].

Discussion

In this study, three new species of *Candolleomyces* are being introduced using morpho-anatomical and phylogenetic analyses. It is very interesting that all new species, *Candolleomyces crenatus*, *C. undulatus*, and *C. virgatus*, formed separate branches from their closest species. Morphologically, *Candolleomyces crenatus* is distinguished from its closest species, *C. badiophyllus*, due to its convex to plane, crenate cap margins, wrinkled, dull surface, light gray, with a dull orange center of pileus, a marginate base of stipe, and small basidiospores (7–9 × 4–5 µm). *Candolleomyces badiophyllus*, a French species, differs from new taxon due to its subglandiform to bell shaped, ochreous yellow, silken, shiny surface of pileus, bulbous base of stipe, and larger basidiospores (11–14.5 × 5–6.2 µm) (Romagnesi 1952).

Candolleomyces undulatus differs from its sister species, *C. efflorescens*, due to its parabolic to campanulate pileus, light reddish gray lamellae, brown basidiospores, terrestrial, connate, and its occurren under *Phoenix dactylifera*. While *C. efflorescens*, from Sri Lanka, differs due to its hemispleuric pil-

eus, white lamellae, and brown-purple basidiospores. It occurred on dead mossy trunks under *Peradenya ceylon* (Saccardo 1887). The second closest species, *C. albipes*, from Jamaica, differs due to its convex to broadly convex, incurved margins of pileus, clavate cheilocystidia, and absence of caulocystidia (Desjardin and Perry 2016). Similarly, *C. tuberculatus*, a French taxon, differs from our newly described species due to its globose, greenish, and loaded with scales in the shape of small rounded tubercles on the pileus, membranous annulus, and purple, ovoid basidiospores (Patouillard 1885–1903).

According to the phylogenetic analyses (Fig. 2), *Candolleomyces virgatus* forms a separate branch from *C. amygdaliformis* and *C. campanulatus*. Morpho-anatomically, *C. virgatus* is different due to its parabolic to plane shape, distinct umbo, virgate surface of pileus, crinkled, 1–7 tiers of forking lamellae, marginate base, longitudinal striations on the surface of the stipe, and lageniform with moniliform at the top, moniliform, cylindrical, tibiiform, and conical cheilocystidia. While *C. amygdaliformis* is different from *C. virgatus* by its conical pileus with absence of umbo, 1–3 tiers of lamellae, shiny surface of stipe, amygdaliform to phaseoliform basidiospores, and ovoid-pedunculate cheilocystidia (Ahmad et al. 2024). *Candolleomyces campanulatus* differs from *C. virgatus* by its conical to hemispheric, indistinct umbo, fibrillose, and plain dry surface of pileus, eroded, 1–2 tiers of lamellae, crystals, or mucoid droplets at the apices of cheilocystidia (Izhar et al. 2023).

These findings show that the diversity of the genus *Candolleomyces* in Pakistan is much more than expected. Hence, many more systematic surveys are required to explore and understand the diversity and distribution the genus.

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Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

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Author contributions

Conceptualization: MH & ANK. Data curation: MH & ARN. Formal analysis: MH & ANK. Investigation: MH & QL. Methodology: MH & ARN. Resources: MH & QL. Software: MH.

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Data availability

All of the data that support the findings of this study are available in the main text or Supplementary Information.

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Supplementary material 1

Fasta file of phylogenetic data

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Data type: fas

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